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Development of a New Kisspeptin Based Method of Ovulation Synchronization for Crossbred Dairy Heifers

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Abstract

The aim of the present study was to develop a new method of synchronization of estrus/ovulation for crossbred cows based on kisspeptin, a potent secretagogue of GnRH. For the purpose, a total of 108 estrous cycles were studied in cyclic heifers. The animals divided equally in to two groups were treated either with ovsynch protocol of estrus synchronization (group-I; GnRH: day 0; PGF2α: day 7 and GnRH: day 9) or with a new method of synchronization based on kisspeptin (group-II; kisspeptin: day 0; PGF2α: day 7 and kisspeptin: day 9). Heifers were monitored regularly with transrectal ultrasonography for follicular dynamics and occurrence of ovulation. It was found that the kisspeptin based protocol induced better growth of follicles than ovsynch one. Ovulation rate was significantly higher (P<0.05) in the animals of group-II than group-I. As revealed through ultrasonography as well as plasma progesterone profiles, the process of luteolysis starts early even before PGF2α injection in kisspeptin based protocol than ovsynch. In conclusion, we developed a new method of synchronization of estrus/ovulation based on kisspeptin in bovine species for the first time. The newly developed method is found to be better than the conventional ovsynch method in terms of percent ovulation in the treated animals.

Keywords: *Estrus, ovulation, synchronization, kisspeptin, metastin, bovine*

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INTRODUCTION

Synchronization programs have become standard components in the current breeding management of cows in dairy herds of most countries worldwide. Many are based on protocols that allow timed inseminations (TAI) so as to circumvent the practical difficulties associated with estrus detection. Artificial insemination (AI) also allows for genetic improvement of replacement dairy heifers and enhances the value of pregnant heifers. Estrus synchronization protocols are based mainly on GnRH and/or PGF2α or their combination thereof. Undoubtedly, the GnRH or its analogues are on the top priority for this purpose.

Efforts of regulation and control of follicular dynamics using GnRH have been made in cattle [1–2]. Ovulation synchronization using GnRH [1] followed by TAI have been reported to be satisfactory in terms of conception rates [3].

In the ovsynch protocol, intramuscular administration of GnRH irrespective of the day of estrous cycle results in induction of a LH peak that promotes ovulation of follicles with diameter more than >9.0 mm [4] or it causes luteinization of nonviable follicles, and thereafter there is emergence of a new follicular wave after two or three days [5–6]. After ovulation or luteinization of the dominant follicle, blood concentrations of progesterone increases and so administration of PGF2α is required to induce luteolysis [5–6]. Following PGF2α, a second dose of GnRH is given after 48 h to confirm ovulation [2, 7].

Presently, the ovulation synchronization protocols like ovsynch as mentioned above (d 0 GnRH, d 7 PGF2α, d 9 GnRH, d 10 timed AI; [8] and Co-Synch (TAI performed at the same time as the second GnRH injection; [5] are commonly used. Recently, reduction of the interval between the initial GnRH and the PGF2α injections from 7 to 5 d (5 d Cosynch

program) was shown to improve pregnancy rates in lactating dairy cows [5]. In common protocols for fixed time insemination, such as ovsynch, a primary GnRH injection is given at some predetermined time after calving, followed by PGF2α 7 d later (the time when the corpus luteum (CL) becomes maximally responsive to PGF2α). An additional injection of GnRH is given 48 h after injection of PGF2α to synchronize ovulation. Because of the time required for CL induced following the first GnRH injection to become responsive to PGF2α, there is less flexibility in control of timing of ovulation of the newly developed dominant follicle relevant to insemination.

Inability to tightly control responsiveness of the CL to PGF2α has additional adverse effects. Incomplete luteal regression following injection of PG is associated with conception failure in the ovsynch protocol [9–11]. A likely major causative factor in conception failure is stage of maturation of the preovulatory follicle in relation to estrus. Pregnancy rates in dairy cattle decline as the interval between ovulatory follicle emergence (or dominance) and subsequent estrus increases [12]. Total regression of the CL is a component of reproductive management that limits pregnancy rates in lactating dairy cows. Therefore, there is need of a protocol that is better than the existing ovsynch protocol where CL has to be developed in such a stage so that it is sensitive to PGF2α on day 7 or even before of the protocol.

Recently, kisspeptin has been reported to be a potent secretagogue of GnRH and play important roles in the regulation of reproduction in animals [13]. Kisspeptin (a product of the KiSS1 gene) is a decapeptide acts mainly through controlling GnRH secretion in the brain [13–14]. Kisspeptin has been found to induce preovulatory LH surges more efficiently than gonadotropins with a very low dose [15]. Hence, it is expected that administration of kisspetin rather than GnRH in the protocol may initiate early ovulation and so earlier development of CL. Therefore, the aim of the present study was to develop a new method of synchronization protocol based on kisspeptin for dairy heifers.

MATERIALS AND METHODS

Animals

A total of 18 crossbred cyclic heifers were selected from the dairy farm of ICAR-National Dairy Research Institute, Eastern Regional Station, Kalyani, West Bengal, India. A total of 108 estrous cycles were studied. A gap of two consecutive cycles was given, if more than one estrous cycle has been studied in the same animal. All animals were fed with the standard feeding practices as followed in the institute herd. Standard institutional managemental practices for heifers were followed. All the experimental methods were approved by the institutional research council.

Ultrasonographic Examination of the Experimental Animals

Follicular dynamics of the experimental heifers were studied using ultrasound machine (DIGI 1100 CD-E Vet, SS Medical, Lucknow, India) and a linear rectal probe of 7.5 to 10.0 MHz frequency.

Experimental Design

All experimental heifers were divided into two groups. In group-I, heifers were treated with ovsynch protocol of estrous synchronization i.e., GnRH on day 0 (any day of the estrous cycle), PGF2α on day 7 and another dose of GnRH on day 9 (n=54 estrous cycles). Animals of group-II were treated with a new method of synchronization based on kisspeptin i.e., kisspeptin on any day irrespective of the day of estrous cycle (day 0), PGF2α on day 7 and another dose of kisspeptin on day 9 (n=54 estrous cycles; (Figure 1). All animals irrespective of the group were observed for ovulation through ultrasonography. The doses of GnRH to the animals of group-I were 20 µg GnRH per animal intramuscular (Busereline acetate, Intervet, India). Intramuscular dose of 10 mg PGF2α (dinoprost tromethamine, Lutalyse®, Pfizer Animal Health, India) was used in the protocol. An amount of 200 µg of kisspeptin 10/metastin (45–54), a decapeptide with C-terminally amidated LRF-motif, (EMD Millipore Corporation, Calbiochem, 290 Concord Road, Billerica, MA 01821, United States of America; Cat. No. #445888) was administered intravenously to each animals of the group-II on day 0 and 9. Ultrasonography was carried out twice-a-day from the beginning of the experiment until the time of ovulation.

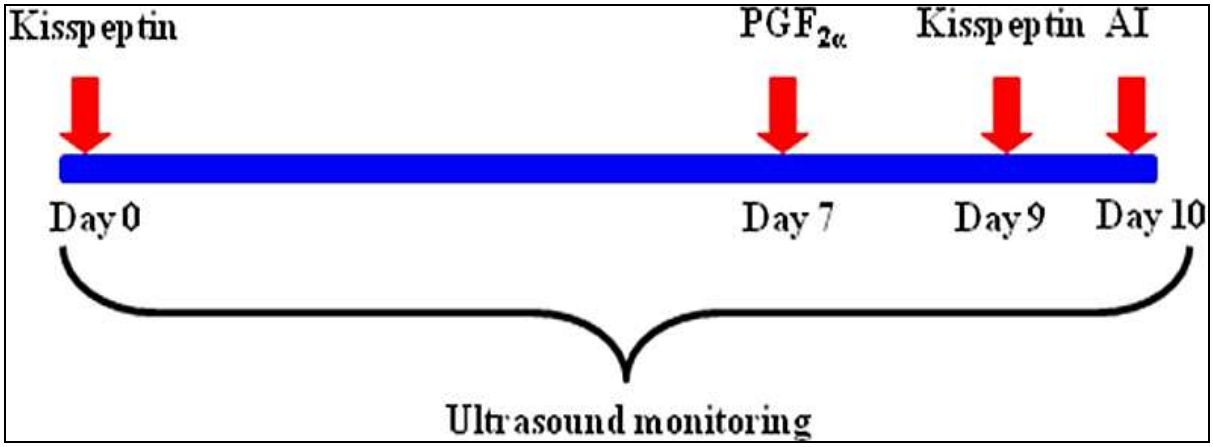

Fig. 1: *Protocol of Synchronization Based on Kisspeptin.*

Blood Sample Collection and Hormonal Analysis

Blood samples were collected daily throughout the experimental period from the jugular vein of each animal in to the tubes containing heparin sodium salt (20 IU/ml). Blood samples were centrifuged at 3000 rpm for 15 min at 4°C. Plasma was separated and stored at −20°C until assayed for plasma progesterone. The samples were analysed for progesterone [16].

Statistical Analysis

All data were expressed as mean±S.E.M. Chi-square test were used to analyze the binomial variables, the percentage of heifers that ovulated after second injections of GnRH or kisspeptin, the percentage of heifers having plasma progesterone concentration less than 1.0 ng/ml and follicle diameter more than 8.0 mm at the time of the treatment. Analysis of variance technique (ANOVA) was used to assess the continuous variables (follicle size and progesterone concentration) for repeated measure. The t-test was also used to evaluate the differences between means. Significance was considered at $P<0.05$ level if otherwise not stated. GraphPad Prism 4.01 software (San Diego, USA) was used for statistical analysis of the data.

RESULTS AND DISCUSSION

Ovarian Ultrasonography for Follicular Dynamics

The occurrence of ovulation in different estrous cycles after second administration of either GnRH or kisspeptin during two protocols of synchronization has been presented in Figure 2. As revealed by ovarian ultrasonography, the ovulation rate post- 2nd GnRH and -kisspeptin administration in the ovsynch and newly developed kisspeptin based protocol of estrus synchronization was 92.6% (50/54) and 98.1% (53/54), respectively. The groups differ statistically ($P<0.05$).

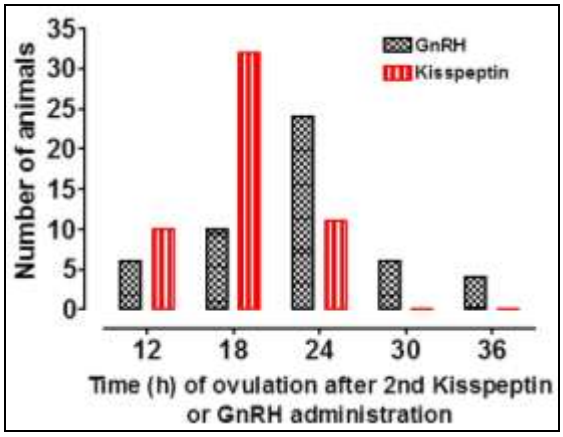

Fig. 2: *Time of Ovulation after Second Injection of GnRH or Kisspeptin.*

In around 60% of the animals of group-II (32/54) ovulated at 18 h post second kisspeptin injection. Around 20% ovulation has been recorded each time at 12 and 24 h post kisspeptin (Figure 2). The time of ovulation ranged between 12 and 24 h. On the other hand, ovulation time ranged between 12 and 36 h post second GnRH injection in the animals of group-I, and majority of ovulation (~50%) was at 24 h after second GnRH administration. In the animals of group-I, the mean diameter of the follicle at the beginning of the experiment was 11.0±0.2 mm. The animals those ovulated post-2nd GnRH

injection exhibited larger follicle diameters (P<0.05) than those reported as anovulatory (11.1±1.5 versus 8.1±1.2 mm). On the other hand, the mean diameter of the largest follicle (dominant follicle) of the animals that ovulate was 11.9±1.3 mm on day 7 and 13.8±1.1 mm on day 9.

Similarly, animals of group-II had the follicles of 10.8±0.3 mm mean diameter at the beginning of the experiment. The animals those ovulated post-2nd kisspeptin injection exhibited larger follicle diameters (P<0.05) than those reported as anovulatory (12.2±1.6 versus 9.6±1.4 mm). On the other hand, the mean diameter of the largest follicle (dominant follicle) of the animals that ovulate was 12.9±1.3 mm on day 7 and 14.9±1.4 mm on day 9 of the treatment.

Progesterone Profiles of GnRH (Ovsynch) vis-a-vis Kisspeptin Based Protocol during Different Days of Treatment

The mean plasma progesterone concentrations were 3.90±0.89 versus 3.20±1.10, 2.40±0.87 versus 3.50±0.98 and 1.90±0.56 versus 2.50±0.80 ng/ml on days 0, 4 and 7 in group II and I, respectively. When PGF2α was administered on day 7, plasma progesterone concentrations started declining to reach the basal level on day 9 of the treatment in animals of both the groups (Figure 3). This is to be noted that the plasma progesterone concentrations of kisspeptin-treated heifers (group-II) started decreasing before administration of exogenous PGF2α and this may probably due to initiation of luteolysis long before PGF2α injection (Figure 3).

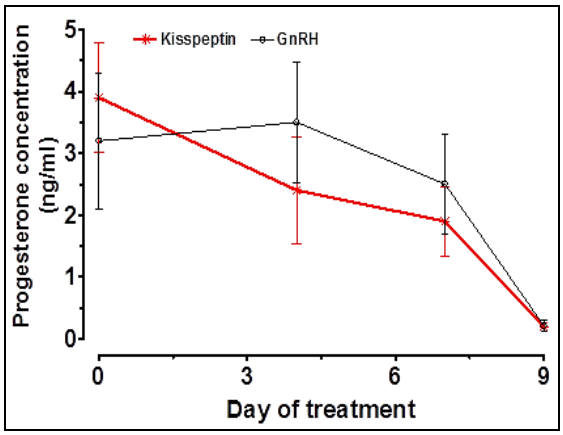

Fig. 3: *Mean Progesterone Concentrations on Different Days of the Protocol Based Either on GnRH (n=50) or Kisspeptin (n=54).*

To the best our knowledge, new synchronization protocol based on kisspeptin as developed in the present study is the first report of this kind in bovine species. Our results showed that GnRH based protocol though responded well (ovulation rate: 92.6%), our developed method based on kisspeptin is better as it induces ovulation in almost all animals treated (ovulation rate: 98.1%). The response of the ovsynch protocol as found in our experiments is comparable with the available reports in bovines [4–5]. It is interesting to note that the plasma progesterone concentrations decreased more rapidly even before PGF2α injection in the heifers of group-II (kisspeptin based protocol) than the ovsynch one. This is due to faster ovulation of dominant follicle on day 0 of the treatment and hence early formation of CL in the group I than group II. Kisspeptin being potent secretagogue of GnRH [13], it causes early ovulation of the dominant follicle thereby faster maturation of CL, which may probably results the kisspeptin based protocol perform better than conventional ovsynch one.

CONCLUSIONS

In conclusion, we developed a new method of synchronization of estrus/ovulation based on kisspeptin in bovine species for the first time. The newly developed method is found to be better than the conventional ovsynch method in terms of percent ovulation in the treated animals.

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